*J Toxicol Pathol 2010; **23**: 165–169*

Short Communication

Histopathological Features of Cultured Japanese Amberjack *Seriola quinqueradiata* with Ascites Occurring in the Winter

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Abstract: A winter outbreak of ascites in cultured Japanese amberjack *Seriola quinqueradiata* was examined. Affected fish displayed bloody ascitic fluid in the peritoneal cavity, their hearts were covered with a white membrane, their livers were congested and their kidneys were swollen. Renal tubular epithelia displayed extensive necrosis and often contained hyaline droplets, while some glomeruli showed atrophy and necrosis. Hematopoietic tissue in the kidney contained some swollen reticular cells. In the heart, an inflammatory reaction accompanied by edema, hemorrhage and fibrin deposition occurred in the epicardium of the atrium and ventricle and in the pericardial cavity. The epicardium and pericardium were partially adhered to each other. Hepatic lesions included dilatation of sinusoids accompanied by severe edema and congestion. Edema and fibrin deposition were often observed in the hepatic serosa and perivascular tissue. Splenic tissues with atrophied pulp showed definite increases in trabecula thickness. These findings suggest that the examined fish might have a systemic circulatory disturbance due to cardiac lesions associated with renal failure. (J Toxicol Pathol 2010; **23**: 165–169)

Key words: Japanese amberjack, *Seriola quinqueradiata*, ascites, histopathological change, renal failure

Japanese amberjack (referred to as "yellowtail" in previous studies) *Seriola quinqueradiata* is an economically important marine fish species in the western part of Japan. Disease is one of the impediments in cultured fish production at the commercial level. Mass mortalities due to infectious diseases have often occurred in Japanese amberjack, and many diseases have been reported[1–3]. In February 2005, mass mortality occurred at a Japanese amberjack farm in Nagasaki Prefecture, which had experienced some similar cases in previous winter seasons (period of low water temperature). However, the cause of death is uncertain, and histopathological examination has not been performed on the affected fish. This paper describes the histopathological features of diseased Japanese amberjack with ascites occurring in the winter.

A total of 6 moribund or freshly dead Japanese amberjacks were collected from those that died in Nagasaki Prefecture in February of 2005. The body weights of the examined fish ranged from 1,050 to 1,180 g, and their body lengths ranged from 37.6 to 38.0 cm (Table 1). Wet mounts of gill biopsies and skin scrapings were examined for ectoparasites with light microscopy. After necropsy, isolation of bacteria from the liver, kidney, spleen and brain was performed on three of the 6 examined fish using modified trypto-soya agar plates[4] at 25°C. The remaining three fish were used for histopathological examinations (Table 1).

The tissues including the brain, gill, heart, liver, spleen, kidney, pancreas, digestive tract, and lateral musculature of 3 examined fish were fixed in phosphate-buffered 10% formalin solution, embedded in paraffin and sectioned at 4 μm. Tissue sections were stained with hematoxylin and eosin (H&E), Giemsa, Periodic acid-Schiff (PAS) reaction, Azan and Phosphotungstic acid hematoxylin (PTAH).

Mass mortalities of cultured Japanese amberjack occurred when the water temperature ranged from 12 to 13°C. The cumulative mortality rate reached about 10 to 15%. Table 2 shows the gross pathological findings in the examined fish. Diseased fishes showed abnormal behavior and loss of body balance and swam in circles at the water surface. The fishes displayed pale body coloration and a distended abdomen. At necropsy, there was excessive mucus on gills. Red spots or redness were observed on the body surface, internal surface of the opercula and in abdominal adipose tissue. The examined fish had bloody ascitic fluid in their peritoneal cavities, congested livers, hearts having a white membrane and swollen kidneys (Fig. 1A). The brains showed redness. The lesions were observed in almost all of the examined fishes. None of the fish had distinct lesions in

Received: 13 January 2010, Accepted: 30 April 2010
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Table 1. Examined Fish in the Present Study

Fish	1	2	3	4	5	6
Body weight (g)	1,180	1,050	1,100	NE	NE	NE
Length (cm)	37.8	37.6	38	NE	NE	NE
BI[1]	Negative	Negative	Negative	NE	NE	NE
Histopathology	NE	NE	NE	E	E	E

NE: not examined. E: examined. [1]: Bacterial isolation test for the brain, kidney, spleen and liver.

Table 2. Gross Pathological Findings of the Examined Fish

Organs	Findings	No. of fish		
		1	2	3
Brain	Redness	++	++	++
Gill	Exclusive mucus	+	+	+
	Redness	–	+	–
Body surface and opercula	Red spot	++	++	++
Heart	White membrane	++	++	++
Kidney	Swelling	++	+	++
Liver	Congestion	+	+	+
Abdominal cavity	Ascites	++	++	++
	Redness[1]	++	++	++
Other organs[2]		–	–	–

Grades: –, + and ++ represent no change, slight and marked, respectively. NE: not examined. [1] In the abdominal adipose tissue. [2] Spleen, digestive organs, fins and eyes (Benedenia-like parasites were slightly observed in eyes of No. 2).

the spleen, digestive organs, fins and eyes. Although a few Benedenia-like monogenean parasites were observed on the body surfaces of all of the fish examined, gill biopsies did not reveal the presence of ectoparasites. In addition, no bacteria were isolated from the livers, kidneys, spleens or brains.

Table 3 presents the main histopathological findings observed in this study. In the kidney, many renal tubular epithelia were necrotized (Fig. 1B), vacuolated or atrophied and were separated from the basement membranes. The necrotic tubular epithelia often contained small hyaline droplets and displayed cloudy swelling. The tubules also contained epithelial casts and/or acidophilic bodies. Glomeruli were slightly atrophied and accompanied by slight fibrin deposition. Hematopoietic tissue contained some swollen reticular cells (Fig. 1B). These lesions were observed in all of the examined fishes.

In the heart, slight inflammatory cellular infiltration accompanied by fibrin deposition and edema were observed in the epicardium of the atrium and ventricle and in the pericardial cavity (Fig. 1C). The fibrin deposition and edema were often observed around the bulbus arteriosus. The epicardium with fibrin deposition and edema contained some necrotic cells with karyopyknosis. The coronal vessels were dilated. The epicardium and pericardium were partially adhered to each other, which might be due to the occurrence of the fibrin deposition and edema in the pericardial cavity. These cardiac lesions were observed in all of the examined fishes. However, no significant histopathological change was observed in the cardiac muscle fibers.

In the liver, the hepatocytes displayed decreased amounts of glycogen, while the hepatic vein displayed congestion. In severe cases, hepatic lesions were characterized by dilatation of sinusoids accompanied by edema and congestion, while many necrotic hepatocytes with karyopyknosis were observed around the dilated sinusoids (Figs. 1D & E). The edema and fibrin deposition were observed in the perivascular tissue (Fig. 1F). Fibrin deposition and edema were often observed in the hepatic serosa with necrotic cells. In cases with low-grade hepatic lesions, the hepatocytes were characterized by atrophy. Fish that showed distinct cardiac lesions possessed severe hepatic lesions.

In the spleen, the splenic pulp was depleted, and the trabeculas were thickened. The splenic lesions were observed in all of the examined fishes. Blood vessels of the splenic tissues often displayed congestion. Cells of the serosa were often necrotic with pyknotic nuclei. This was in conjunction with subserosal fibrin deposition.

In some fish, the submucosa of the stomach displayed extensive edematous dissociation. In gills, there was slight hyperplasia in the epithelial cells at the top of the gill lamellae. Furthermore, a dilatation of the blood vessels was observed in the dermal tissue. No lesions were observed in other organs, including the lateral musculature, pancreas, abdominal adipose tissue and brain. No bacterial infection

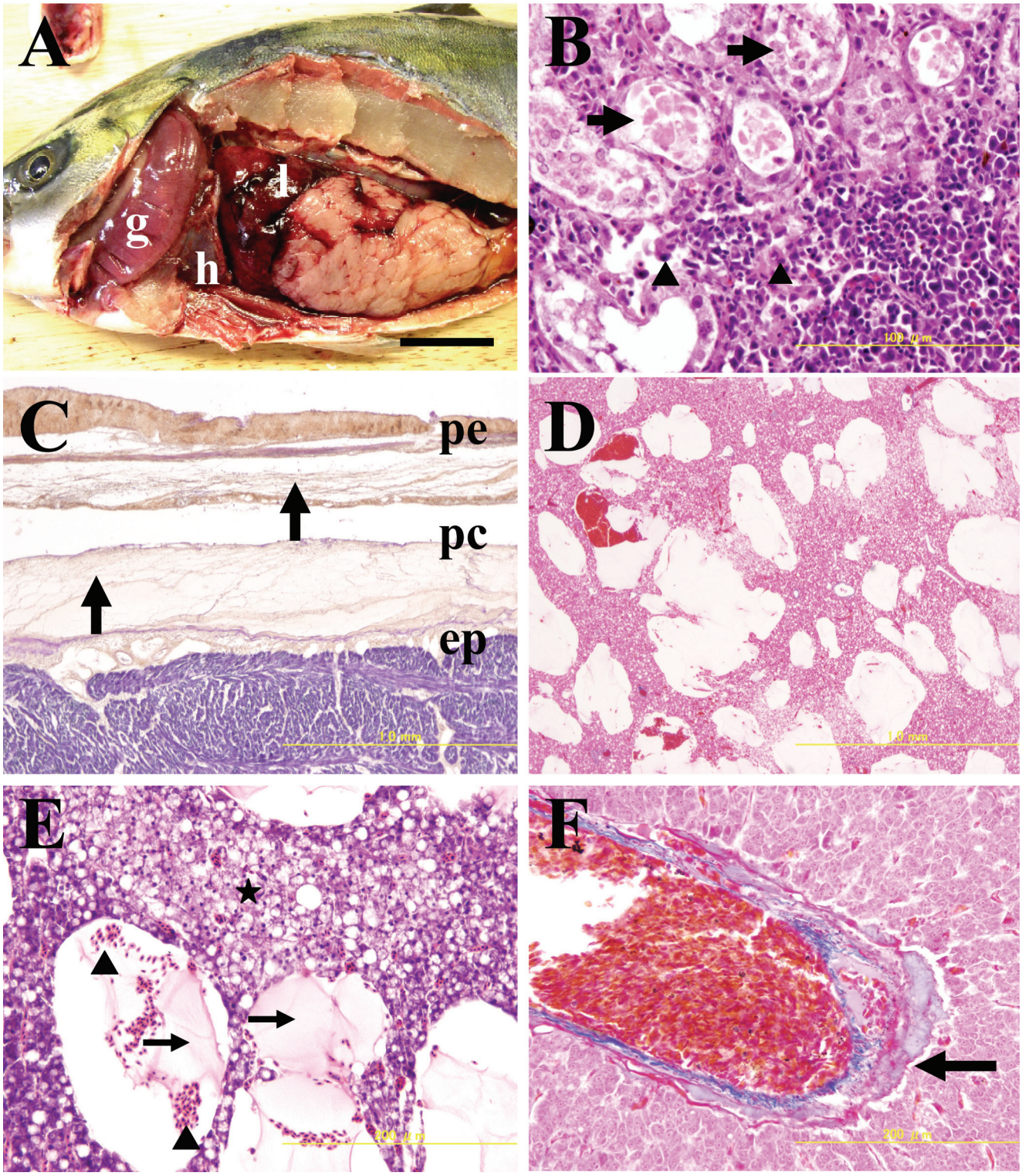

Fig. 1. A: Diseased fish with pale coloration of the gills, a heart with a white membrane and a congested liver. The scale bar indicates 3 cm. g: gill. h: heart. l: liver. B: Histopathological features of the kidney. Renal tubular epithelia are necrotized (↑). Some swollen reticular cells (▲) are observed in the hematopoietic tissue. H&E. C: Histopathological features of the heart. Severe fibrin deposition (↑) occurs in the epicardium and the pericardial cavity (pc). PTAH. ep: epicardium. pe: pericardium. D: Histopathological features of the liver. The hepatic parenchyma displays many vacuolated spaces. Azan. E: Histopathological features of the liver. Dilated sinusoids contained many erythrocytes (▲) and eosinophilic materials (↑). Necrotic lesions (★) were also observed around the dilated sinusoids. H&E. F: The hepatic vein shows congestion, while edema and fibrin deposition (↑) occur in the perivascular tissue. Azan.

Table 3. Main Histopathological Features of the Examined Fish

Organs	Findings	No. of fish		
		4	5	6
Gill	Hyperplasia of epithelial cells	–	+	+
Heart and Bulbus arteriosus	Fibrin deposition, pericardial cavity	+++	+++[1]	++
	Hemorrhage and edema, pericardial cavity	+++	++	+
	Epicarditis	+++	++	++
Kidney	Tubular necrosis	+++	++	++
	Vacuolization of tubular epithelium	++	+	+++
	Hyaline droplets and/or cloudy swelling, tubular epithelial cells	+	+	+
	Urinary cast	++	++	++
	Atrophy, glomeruli	+	+[2]	++
	Swollen reticular cells, hematopoietic tissue	++	+	+++
Liver	Dilatation[3], sinusoids	+++	++	+++
	Edema and fibrin deposition, perivascular tissue	+	+	+
	Atrophy, hepatocytes	–	++	++
	Edema and fibrin deposition, serosa	+++	++	+
Spleen	Atrophy, splenic pulp	+++[2]	+++	+++
Stomach	Edema, muscle layer	++	–	NE
Other organs[4]	Significant lesions	–	–	–

Grades: –, +, ++ and +++ represent no change, slight, marked and severe, respectively. NE: not examined.
[1] Accompanied by adhesion of epicardium and pericardium. [2] Accompanied by fibrin deposition. [3] Accompanied by edema and congestion. [4] Skin, muscle, pancreas and intestine (skin and muscle: data only of No. 6).

was observed in the lesions of visceral organs or gills.

The most marked pathological features were seen in the kidneys presenting severe tubular necrosis and in the hearts in conjunction with fibrinous pericarditis and resulting adhesion to the pericardium and/or edema of the pericardial cavity. In mammals, fibrinous pericarditis often occurs in association with renal failure, such as with uremia; however, the mechanism is not well understood[5]. In the present study, there were similarities to cases described in mammals regarding the relationship between renal and cardiac lesions. Moreover, the fibrin deposition and ascites likely resulted from circulatory disturbance due to cardiac failure. Thus, the pathological findings indicated that the examined fish were likely in a shock state due to a circulatory disturbance.

Similar diseases with ascites have been reported in cases of viral infections caused by yellowtail ascites virus (YATV) in yellowtail fingerlings[2] and viral hemorrhage septicemia virus (VHSV) in flounders[6]. YTAV-infected yellowtail showed necrotic foci in the liver and pancreas, whereas no obvious histopathological change was observed in the heart tissues[1,7]. There were marked necrotic lesions in the cardiac muscle fibers of the VHSV-infected flounders. On the other hand, a non-infectious disease in yellowtail has been reported to be caused by nutritional myopathy syndrome consisting of a severe degeneration of the lateral musculature along with a visceral ceroidosis[8]. However, such changes were not prominent in the present study. Thus, the present case shows unique histopathological features.

Surprisingly, the present case has been diagnosed as "cold-water stress" because the disease had always occurred in the winter season (period of low water temperature)[9]. The optimum water temperature for yellowtail has been reported to be in the range of approximately 20 to 30°C[10]. The histopathological features of fish cold-water temperature stress have been reported in one case of Japanese eel, *Anguilla japonica*. The histopathological features of the examined eels exposed to cold water (15°C) were characterized by nephrotic changes such as cloudy swelling and hyaline droplet degeneration of some renal tubular epithelia[11]. The lesions were similar to those of the present case. However, cardiac lesions as seen in the present case were not observed in the eels, and none of the eels died. Thus, the present case seems to be a more severe case than that of the eels. In conclusion, it is possible to suspect that maladaptation due to "cold-water stress" may be related to the occurrence of the present case, although the etiology remains obscure.

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